



RECEIVED  
U.S. PATENT AND TRADEMARK OFFICE  
SEPTEMBER 16, 2003  
1615  
P 081-0113P  
TECH CENTER 1600/2900

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: JORGENSEN et al. Conf.: 3281  
Appl. No.: 09/781,893 Group: 1615  
Filed: February 9, 2001 Examiner: KISHORE, G.S.  
For: LIPID-BASED DRUG DELIVERY SYSTEMS CONTAINING  
PHOSPHOLIPASE A2 DEGRADABLE LIPID DERIVATIVES AND  
THE THERAPEUTIC USES THEREOF

LETTER

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

September 10, 2003

Sir:

Under the provisions of 35 U.S.C. § 119 and 37 C.F.R. § 1.55(a), the applicant(s) hereby claim(s) the right of priority based on the following application(s):

<u>Country</u>	<u>Application No.</u>	<u>Filed</u>
DENMARK	PA 2000 00211	February 10, 2000
DENMARK	PA 2000 00616	April 12, 2000

A certified copy of the above-noted applications is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fee required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By

Leonard R. Svensson, #30,330

P.O. Box 747  
Falls Church, VA 22040-0747  
(703) 205-8000

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail, postage prepaid, in an envelope to:  
Commissioner for Patents, P.O. Box 1450, Alexandria,  
VA 22313-1450, on: Sept 10, 2003  
(Date of Deposit).

**BIRCH, STEWART, KOLASCH & BIRCH, LLP**

Lillian Deneen Worthy  
(Signature)  
Sept. 10, 2003  
(Date of Signature)

LRS/KR/sml  
Attachment(s)  
2081-0113P

(Rev. 04/29/03)



# Kongeriget Danmark

Patent application No.: PA 2000 00211

Date of filing: 10 February 2000

Applicant:  
(Name and address)  
Kent Jørgensen  
Bagsværd Hovedgade 99, 12-H  
2880 Bagsværd  
Denmark

Title: Lipid-based microcarrier drug-delivery systems containing phospholipase A2 degradable lipid agents and the therapeutic uses thereof.

IPC: -

This is to certify that the attached documents are exact copies of the above mentioned patent application as originally filed.



Patent- og Varemærkestyrelsen  
Økonomi- og Erhvervsministeriet

28 August 2003



John Nielsen



LIPID-BASED MICROCARRIER DRUG-DELIVERY SYSTEMS CONTAINING PHOSPHOLIPASE A2  
DEGRADABLE LIPID AGENTS AND THE THERAPEUTIC USES THEREOF

---

INVENTORS: KENT JORGENSEN, JESPER DAVIDSEN, CHARLOTTE VERMEHREN,  
SVEN FROKJAER & OLE G. MOURITSEN.

Department of Pharmaceutics, The Royal Danish School of Pharmacy,  
2100 Copenhagen and Department of Chemistry, Technical University of Denmark,  
2800, Lyngby

---

**ABSTRACT AND SUMMARY**

The principle of drug targeting, release and absorption by endogenous phospholipase A2 (PLA2) which is illustrated in Fig. 1a, can be applied to a case also involving lipid-based prodrugs. In this case lipid-analogue compounds are incorporated into the carrier liposome and act as prodrugs which are turned into active drugs by hydrolysis via the endogenous PLA2 that is present in elevated concentrations in the diseased target tissue. This principle including a lipid-based prodrug is schematically illustrated in Fig. 1b. A specific example is a prodrug of a certain mono-ether lipid which exhibits anti-cancer activity. Also included is a therapeutic active compound that is ester bound to the phospholipid in the sn-2 position and therefore is a substrate for PLA2. If the mono-ether lipids are modified with, e.g. a ester-linked derivative in the sn-2-position and therefore can be hydrolysed by PLA2 at the target site, these modified mono-ether lipids constituting the carrier liposome will act as prodrugs that become hydrolysed and turned into drugs by PLA2 at the target site. In this way therapeutically active substances, e.g., monoether lipids and ester-linked derivatives become generated at the desired target site. Pharmaceutical compositions containing the lipid-based microcarriers can be used therapeutically, for example, in the treatment of cancer and inflammation.

**FIELD OF THE INVENTION**

The invention involves lipid-based pharmaceutical compositions used in the treatment of various disorders, e.g. cancer and inflammatory conditions.

**BACKGROUND OF THE INVENTION**

Mono-ether lyso-phospholipids and alkyl phosphocholines are known to be effective anticancer agents. Several mechanisms of the toxic action of ether-lipids towards cancer cells have been proposed involving lack of alkyl-cleavage enzymes in cancer cells. This leads to accumulation of the ether-lipids in the cell membranes which induce membrane defects. Other potential mechanisms of action include effects on intracellular protein phosphorylation and disruption of the lipid metabolism. Normal cells typically possess alkyl-cleavage enzymes, which enable them to avoid the toxic effect of ether-lipids. However, some normal cells e.g., red blood cells, have like cancer cells no means of avoiding the disruptive effect of the etherlipids. Accordingly, therapeutic use of ether-lipids requires an effective drug-delivery system that protects the cells from the toxic effects.

This invention provides such a delivery system in the form of lipid-based carriers, e.g. liposomes composed of lipid-bilayer forming ether-lipids such as glycerophospholipids containing an

alkyl-linkage in the 1-position and an acyl-linkage in the sn-2-position on the glycerol backbone. In addition, the carrier system may contain lipid-bilayer stabilising components, e.g. lipopolymers and sterols which lead to an increased vascular circulation

time and as a consequence an accumulation in the diseased target tissue. When the carriers reach the target site of therapeutic action, e.g. cancer cells, PLA2-catalyzed hydrolysis of the acyl-linkage releases the therapeutically active components, i.e. lyso-etherlipids and ester-linked derivatives.

Contradictory to alkyl-cleavage enzymes which are nearly absent in cancer cells, PLA2 activity is elevated in cancer tissue. In addition, PLA2 activity is elevated in diseased regions such as inflammatory tissue.

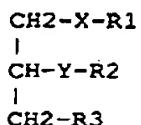
The cytotoxic effects of a broad range of anticancer agents are likely to improve when encapsulated in the carriers of this invention. Furthermore, it is expected that the hydrolysis products, i.e. lyso-monoetherlipids and ester-linked derivatives, act in turn as absorption enhancers for drug permeation across the target membranes when the carriers locally are broken down in the diseased tissue.

#### References:

- (1) New principle for liposomal drug targeting, release and absorption by endogeneous phospholipase A2. J. Davidsen, K. Jorgensen, C. Vermehren, S. Frokjaer, and O. G. Mouritsen (preprint)

#### What is claimed is:

1. A lipid based drug delivery system for administration of an active drug substance selected from the group consisting of a lipid and/or derivatives thereof, wherein the active drug substance is present in the lipid based system in the form of a prodrug and the prodrug constitute the major part of the lipid based system. The lipid has the formula:



X is oxygen O, carbon C, sulfur S. Preferably X is O or C.

Y is  $-\text{OC(O)}-$ , Y then being connected to R2 by way of either the oxygen or carbonyl carbon atom. Preferably R2 is connected by the carbonyl carbon atom. Y can also be  $-\text{NHC(O)}-$ , Y is then being connected to R2 by either the nitrogen or carbonyl carbon.

R1 is an alkyl group, or a halogen-substituted alkyl group.  
R1 is a group having the formula  $\text{Y}_1\text{Y}_2$ ;

$\text{Y}_1$  is  $-(\text{CX}_2)^{\text{n}_1}$   $(\text{CX}=\text{CX})^{\text{n}_2}$   $(\text{CX}_2)^{\text{n}_3}$   
 $\text{Y}_2$  is  $-(\text{CX}=\text{CX})^{\text{n}_4}$   $(\text{CX}_2)^{\text{n}_5}$   $(\text{CX}=\text{CX})^{\text{n}_6}$   $(\text{CX}_2)^{\text{n}_7}$   
 $(\text{CX}=\text{CX})^{\text{n}_8}$   $(\text{CX}_2)^{\text{n}_9}$ ;

X is a halogen or hydrogen, but is preferably hydrogen,  
the sum of  $\text{n}_1+2\text{n}_2+\text{n}_3+2\text{n}_4+\text{n}_5+2\text{n}_6+\text{n}_7+2\text{n}_8+\text{n}_9$  is an integer of from 9 to 29;

n1 is zero or an integer of from 1 to 29, n3 is zero or an

integer of from 1 to 20, n5 is zero or an integer of from 1 to 17, n7 is zero or an integer of from 1 to 14 and n9 is zero or an integer of from 1 to 11;

each of n2, n4, n6 and n8 is independently zero or 1;

Y2 is CH3 or CO2H,

R2 is an alkyl group, or a halogen-substituted alkyl group  
R2 is a group having the formula Y1Y2;

Y1 is -(CX2)n1 (CX=CX)n2 (CX2)n3  
(CX=CX)n4 (CX2)n5 (CX=CX)n6 (CX2)n7  
(CX=CX)n8 (Cx2)n9;

X is a halogen or hydrogen, but is preferably hydrogen,  
the sum of n1+2n2+n3+2n4+n5+2n6+n7+2n8+n9 is an integer of from 9 to 29;

n1 is zero or an integer of from 1 to 29, n3 is zero or an integer of from 1 to 20, n5 is zero or an integer of from 1 to 17, n7 is zero or an integer of from 1 to 14 and n9 is zero or an integer of from 1 to 11;

each of n2, n4, n6 and n8 is independently zero or 1;

Y2 is CH3 or CO2H,

R3 is phosphatidic acid, PO2-OH

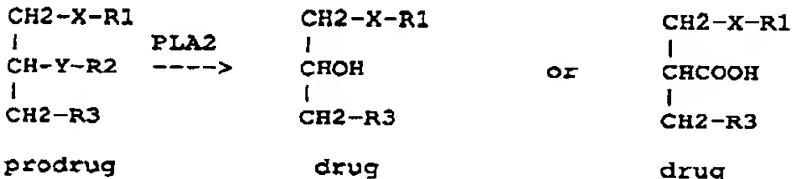
R3 is phosphatidylcholine, PO2-O-CH2CH2N(CH3)3

R3 is phosphatidylethanamine, PO2-O-CH2CH2NCH2

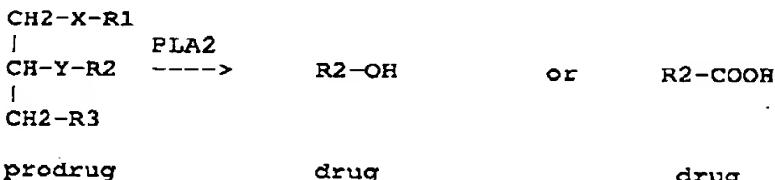
R3 is phosphatidylethanamine with a polymer covalently attached to the terminally positioned N. Suitable polymers include polyethylene glycol (PEG), polyactide, polyglycolic acid, polyactide-polyglycolic acid copolymer, and polymvinyl alcohol all having molecular weight from 100 daltons to 10000 daltons.

R3 is phosphatidylglycerol, PO2-O-CH2CHOHCH2OH

2. The active drug agents released from the prodrug described in claim 1 can be generated in the following way:



and/or



3. A drug delivery system according to claim 1, wherein the lipid based system is in the form of liposomes and wherein the liposomes are build up of layers comprising the prodrugs described in claim 1.

4. A drug delivery system according to claim 1, wherin the lipid based system is in the form of a particulate lipid system.

5. A drug delivery system according to any of claims 1-4, wherein the active drug substance is a monoether lipid selected from the group according to claim 2 where

X is O

R1 comprises alkyl chains  $(CH_2)_nCH_3$  where n is 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, but is preferably 15 or 17

Y is  $-OC(O)-$ , Y then being connected to R1 by way of the carbonyl carbon atom.

being connected to R2 by either the nitrogen or carbonyl carbon.

R2 comprises alkyl chains  $(CH_2)_nCH_3$  where n is 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, but is preferably 15 or 17

6. A lipid based drug delivery system consisting of monoester lipid prodrug according to claims 1-5 comprising mixtures thereof.

7. A drug delivery system according to any of the preceeding claims, wherein the prodrug of the active drug substances are generated as described in claim 2.

8. A drug delivery system according to claim 2, wherein the liposomes are build up of layers consisting of prodrugs of monoether lipids and/or monoesterlipids and/or derivatives thereof.

9. A drug delivery system according to any of the preceeding claims further comprising a compound which stabilise the liposome. The stabilising compound is from the group of lipopolymers (polyethyleneoxide-dipalmitoylphosphatidyl ethanclamine, DPPE-PEG, polyethyleneoxide-distearoylphosphatidylethanclamine, DSPE-PEG) with PEG molecular weight from 100 to 10000 Daltons. The stabilising compound also includes sterols such as cholesterol.

10. A drug delivery system according to any of the preceeding claims further containing a component which controls the release of any bioactive substance which is therapeutically and/or cosmetically active.

11. A drug delivery system according to any of the preceeding claims further comprising phospholipase A2 activity controlling agents.

12. A drug delivery system according to any of the preceeding claims further comprising a permeability enhancer.

13. A drug delivery system according to any of the preceeding claims further comprising a dispersion medium.

14. A drug delivery system according to claim 13, wherein the dispersion medium is a pharmaceutically acceptable aqueous medium.

15. A drug delivery system according to any of the preceeding claims further comprising a therapeutically and/or prophylactically active substance. Active agents include (i) antitumor agents such as anthracyline derivatives, cisplatin, paclitaxel, 5-fluoruracil, and vincristine (ii) antibiotics and antifungals (iii) antiinflammatory agents such as steroids and non-steroids. 16. A drug delivery system according to any of the preceeding claims and adapted to be administered via intravenous and intramuscular injection, and via topical and ocular routes the liposomes having a mean particle size of about 100 nanometer.

17. A drug delivery system according to any of the preceeding claims

and adapted to be administered via subcutaneous injection, the liposomes having a mean particle size from 100 to 5000 nanometers and the liposomes can be uni- or multilayered.

18. A drug delivery system according to any of the preceding claims which is stable towards sterilization by means of e.g. sterile filtration and autoclaving. Preferably no more than 10% degradation of the liposomes is allowed due lipid breakdown.

19. A drug delivery system according to any of the preceding claims which enables the active substance to be delivered to the target within the body.

20. A method for the preparation of a drug delivery system according to any of the preceding claims, the method comprising the steps of:  
(a) dissolving the lipid components in an organic solvent;  
(b) removing the organic solvent from the lipid solution of step (a); and  
(c) hydrating the product of step (b) with an aqueous phase so as to form liposomes. The method further comprises a step of adding an additional bioactive agent to the organic solvent of step (a) or the aqueous phase of step (c).

21. The method of claim 20, further comprising a step of extruding the liposomes produced in step (c) through a filter to produce liposomes of a certain size, e.g. 100 nanometer.

22. The method of claim 20, further comprising the step of loading an additional biactive agent into the liposome by way of electrochemical potential, e.g. pH gradient across the liposome's bilayer.

23. A method for selectively drug targeting to neoplastic cells, e.g., areas within the body having a concentration of phospholipase A2 which is higher compared to normal values by administering to a patient in need thereof an efficient amount of a drug using a drug delivery system described according to any of the preceding claims.

24. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the lipid based drug delivery system according to claim 1.

Patent- og  
Varemærkestyrelsen

10 FEB. 2000

Modtaget

### Liposomal drug targeting, release and absorption principle by endogenous enzymes

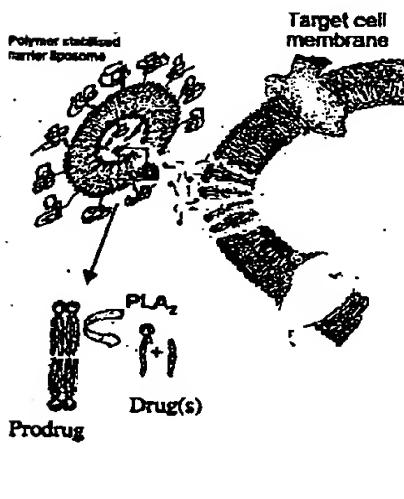
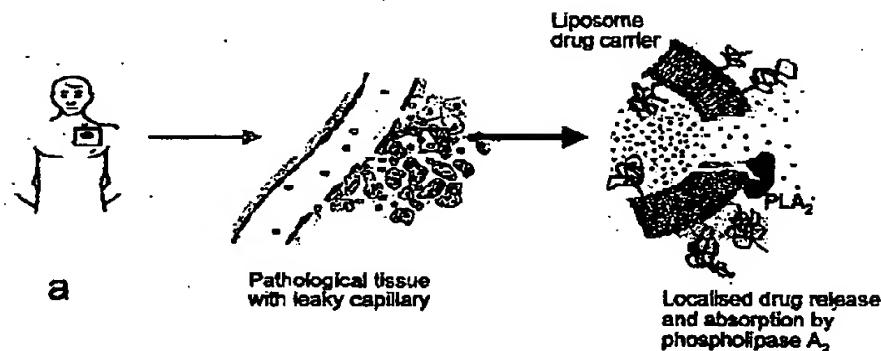


Figure 1